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IN THE UNITED STATES PATENT & TRADEMARK OFFICE

IN RE APPLICATION OF	:
ALAN K. SMITH, ET AL.	: EXAMINER: BELYAVSKI
SERIAL NO: 09/027,671	:
FILED: FEBRUARY 23, 1998	: GROUP ART UNIT: 1644
FOR: HUMAN LINEAGE COMMITTED CELL COMPOSITION WITH ENHANCED PROLIFERATIVE POTENTIAL, BIOLOGICAL EFFECTOR FUNCTION, OR BOTH; METHODS FOR OBTAINING SAME; AND THEIR USES	:

DECLARATION UNDER 37 C.F.R §1.132

COMMISSIONER FOR PATENTS
ALEXANDRIA, VIRGINIA 22313

SIR:

I Douglas M. Smith state that:

I am a named inventor of this application.

I understand that the U.S. Patent Office has rejected the claims of this application in view of U.S. patent no. 5,994,126 and U.S. patent no. 5,290,700 as a basis to allege that the claims of the above-identified application would have been obvious to one of skill in this field.

I disagree that the claims would have been obvious based on what is described in those documents for at least three reasons.

First, neither publication describes or suggests the aspect of culturing T-cells. Indeed, the U.S. '126 patent is focused on culturing dendritic cells (i.e., not T-cells) and the U.S. '700 patent provides a teachings for culturing cells, generally, in the specialized apparatus that is

the subject of that patent. In fact, the only mention of cells in the U.S. '700 patent is in column 2 where the patent describes that the method and apparatus can be used to culture any cell, bacterial, human or other. Culturing a bacterial cell or even a mammalian cell (such as transformed cells that are commonly cultured) have vastly different properties, require vastly different culture conditions and techniques and, in fact, it is well-known that even among cells of a particular species that what one knows about one cell type gives the researcher no *a priori* information as to how cells of a different type will behave in that culture. The U.S. '700 patent also provides no examples of actually culturing cells in its specialized apparatus.

Second, the relevant literature for T-cells describes that the conventional process of culturing is to maintain T-cell concentrations in tissue culture flasks or gas permeable culture bags at an optimum of 10^5 to 10^6 per ml or less based on common knowledge in the field of cell culture. Conventional wisdom suggests that when T-cells achieve a maximum cell concentration of 1-3 million cells per ml, the cultures must be diluted and split immediately into multiple cultures such that density is restored to 5×10^5 cells/ml or less (i.e. "hemi-depletion" to maintain low cell density by adding medium and/or splitting individual cultures into multiple cultures). Typically, this process is repeated several times (generally every 2-7 days during the course of standard T-cell culture (See Riddell and Greenberg, 1990 *J Immunol Methods 128*:189-201).

Third, an individual with skill in the art of T-cell culture would not expect or predict enhanced proliferative potential or cytokine release after culture at high T-cell densities. The opposite would be expected and taught per Ahearn et al. (general case) or '358 June et al. (relating specifically to T-cells) as cited previously by the examiner. In fact, these well-known considerations as related to high-density contact inhibition of T-cell proliferation and other biological functions were our primary concern at initiation of the experiments. Rapid medium exchange under these conditions was expected *at best* to maintain viability and

equivalent functionality. However, there was no reason to believe that continuous high density culture would improve proliferative capacity. Therefore, the results demonstrating strikingly enhanced proliferation at the time of harvest were entirely unexpected.

Note that other artificial capillary culture systems or hollow fiber bioreactors have been used to expand tumor infiltrating T-lymphocytes (TILs) for cancer therapy. However, these systems require inefficient repetitive medium re-circulation to support gas and nutrient exchange. Oxygen and nutrient molecules must diffuse long distances from the fiber lumen to areas of cell growth in the extra-capillary space when compared to the Aastrom system (Mandalam et al. Chapter 13 in: Schindhelm K, Nordon R eds. Ex Vivo Cell Therapy: Academic Press, San Diego, 1999: 273-291). The '700 device is an improvement for gas exchange compared to other hollow fiber systems but may have similar limitations for medium/nutrient exchange. Also, a high inoculum density is required to initiate culture ($>10^8$ TILs required in many hollow fiber systems) and these systems are not optimal for harvest and recovery of the cell product from the extra-capillary fiber bundles. In fact the harvest strategy has been a major limitation for these designs. Harvest would be particularly problematic for the '700 device given the multiple layers of fibers both for gas and medium exchange. The '700 patent and other hollow fiber systems were designed primarily for obtaining cell metabolites or secreted products such as monoclonal antibodies and not the cellular product as provided in the claims of the present application.

I also note the assertion on static culture conditions in the Official Action on page 4 bottom half of page, i.e., "comparison to static culture conditions would be quite expected to one skilled in the art" and top of page 5 "cells growing under static conditions (0% exchange) for more than 7, 12 and 19 days would have different biological function compare to cells grown under conditions of continuously culture exchange"

It should be noted, however, that in the data presented below and the examples presented in the application for T-cell culture that the control flasks are maintained by hemi-depletion (not static) in contrast to the objection raised regarding comparisons to true static cultures (i.e. zero medium exchange). In fact hemi-depletion involves substantial medium exchange as described in the attached methods section for expansion of melanoma tumor reactive T-cells. Thus, direct comparison and superiority of the new methodology to hemi-depletion cultures further increases the unexpected nature of the results demonstrating enhanced proliferative potential and cytokine release.

New data described in the example below shows that patient-derived tumor-reactive T-cells cultured at high density under the medium exchange culture conditions (of the claims) compared to control hemi-depletion methodology express enhanced cytokine secretion. Cytokine release is markedly improved in response to triggering using polyclonal activators such as anti-CD3 monoclonal antibody or specific triggering using melanoma tumor antigens including peptide epitopes or endogenous tumor antigens expressed by a panel of melanoma tumor cells.

These data demonstrate superior cytokine release extending beyond our original example of PHA-activated CD8+ T-cells to include clinically relevant TILs from a melanoma patient. In addition the experiment demonstrates tumor antigen specificity (i.e. the expanded TILs not only secrete higher levels of cytokine using our method but also respond only to tumor-antigen positive targets and not against irrelevant stimulating cells in a melanoma tumor cell panel).

Obtaining T-cells with enhanced proliferative potential and cytokine secretion under these continuous culture conditions is entirely unexpected and counter-intuitive to those skilled in the art as indicated above (i.e. conventional wisdom emphasizes splitting cultures to maintain low density).

EXAMPLE: Expansion of melanoma reactive tumor infiltrating lymphocytes (TILs) under perfusion culture conditions compared to hemi-depletion conditions in tissue culture flasks.

Well-characterized cryopreserved/thawed MART-1 reactive TILs from an HLA-$A2^+$ melanoma patient were expanded in a clinical-scale bioreactor system using preferred methodology as described in the claims for the invention (For an overview of the perfusion bioreactor system see Mandalam et al. Chapter 13 in: Schindhelm K, Nordon R eds. Ex Vivo Cell Therapy: Academic Press, San Diego, 1999: 273-291). Bioreactor cassettes (850 cm^2 PETG tissue culture-treated growth surface) were inoculated at 20 x 10^6 TILs per cassette (6.67 x 10^4 cells/ml) (A) or 5 x 10^6 TILs per cassette (1.67 x 10^4 cells/ml) (B) together with 1 x 10^9 irradiated allogeneic feeder cells (peripheral blood mononuclear cells) in complete medium containing anti-CD3 mAb (OKT3, Orthoclone, 30 ng/mL) and 6000 international units (IU) per ml recombinant human interleukin-2 (rhIL-2) (Chiron Corp., Emeryville, CA). Complete medium (CM) consisted of RPMI 1640 and AIM V (1:1 ratio), 25 mM HEPES, 50 U/mL penicillin, 50 mcg/mL streptomycin, 20 mcM 2-mercaptoethanol, and 5% human AB serum (Valley Biomedical Products and Services, Inc., Winchester, VA). The rate of continuous perfusion for medium containing IL-2 alone was adjusted to maintain a lactate tolerance level of 0.5 - 1.0 mg/ml as determined by sampling and measurement in the waste medium. Small-scale PETG (polyethylene terapthalate-glycol plastic) T-flasks (InVitro Scientific Products, Inc., Ventura, CA) as controls for each bioreactor were established at identical inoculum densities per unit surface area and maintained under conditions mimicking as closely as possible the conditions of the clinical-scale system with respect to medium height (3 mm), gas (20% oxygen environment), and medium exchange rates. A standard low density process for expansion of T-cells (< 1 x 10^6 TILs/ml as maximum T-cell density) in polystyrene T-flasks with manual medium replacement and hemi-depletion steps on days 2, 5, 7, 9 and 12 was included for comparison as an additional control (Riddell and Greenberg,

1990 as adapted by Dudley et al, 2002 and 2005). In contrast to the standard hemi-depletion control group as specified by Riddell, Greenberg and Dudley et al., bioreactors and PETG T-flasks were maintained by perfusing or manually exchanging culture medium based on lactate measurements with subsequent T-cell expansion to high cell density (>19 x 10^6 T-cells/ml) in a continuous process without subculture. For the control process in 25 cm^2 PETG T-flasks, a constant medium volume of 7.5 mls providing a 3 mm liquid height was maintained throughout the entire culture period. Alternatively, a 75 cm^2 PETG T-flask using 22.5 mls medium (also providing a 3 mm medium height) may be used. This medium depth provides conditions for gas exchange using a 20% oxygen enviroment which are comparable to the clinical scale system. In contrast to the continuous replacement of medium in the clinical-scale bioreactor system, medium exchange in the PETG T-flasks was carried out manually as a single replacement at a rate of at least 25-100% per day based on lactate production as described in the claims. In addition, T-cells removed in the spent medium were recovered by centrifugation, resuspended in fresh medium and returned to the PETG T-flasks thus ensuring that no cells were lost from the culture during medium exchange. This latter step was not required at clinical-scale as <1% of T-cells were removed in the bioreactor waste medium as achieved in the bioreactor design. Thus, the T-cell density was not reduced or adjusted at any time during the culture process while maintaining a constant culture volume with frequent medium replacement during progressive T-cell expansion from low to high density.

The cultures were harvested for analysis in assays for cytokine secretion (ELISA) on day 14 (Figure 1). The viability of all cultures was >95% as assessed by hemacytometer count using trypan blue dye exclusion or by flow cytometry using 7-AAD dye exclusion. Cytokine secretion was assessed as described in Dudley et al (2002 and 2005) and the legend of Figure 1. Briefly, 10^5 TILs were incubated in round-bottom microwells (0.2 mls) pre-coated with plastic–immobilized OKT3 mAb. Alternatively, 10^5 TILs were co-cultured with

10^5 irradiated T2 or melanoma tumor cells as indicated in Figure 1. Supernatants were harvested after a culture period of 24 hours and cytokine concentration was determined by ELISA.

As shown in Figure 1-A, GM-CSF and IFN gamma cytokine release in response to immobilized anti-CD3 mAb (polyclonal T-cell receptor triggering) was enhanced under perfusion culture conditions in both small-scale PETG control T-flasks or clinical-scale bioreactor systems when compared to the standard low density hemi-depletion T-flask controls (Figure 1-A). A similar pattern of high antigen-specific IFNgamma release in response to MART-1 peptide loaded T2 cells or against a panel of MART-1-expressing HLA-A2(+)ve melanoma cells (Mel526, TC624) was observed (Figure 1-B). Again, the greatest levels of tumor antigen-specific cytokine release were demonstrated for TILs expanded at high density under perfusion culture conditions compared to TILs produced in standard low density control T-flasks maintained by hemi-depletion. No responses (<5 pg/ml background) were detected against irrelevant g280 peptide-loaded T2 cells or HLA-A2(-)ve MART-1(+)ve melanoma cells (TC938, TC888).

These observations demonstrate that TILs produced under perfusion culture conditions express high functionality and melanoma tumor antigen specificity. These results may reflect the high metabolic activity of T-cells harvested from perfusion cultures or other biological effects of perfusion in combination with the unique microenvironment and cellular interactions produced in high density cultures.

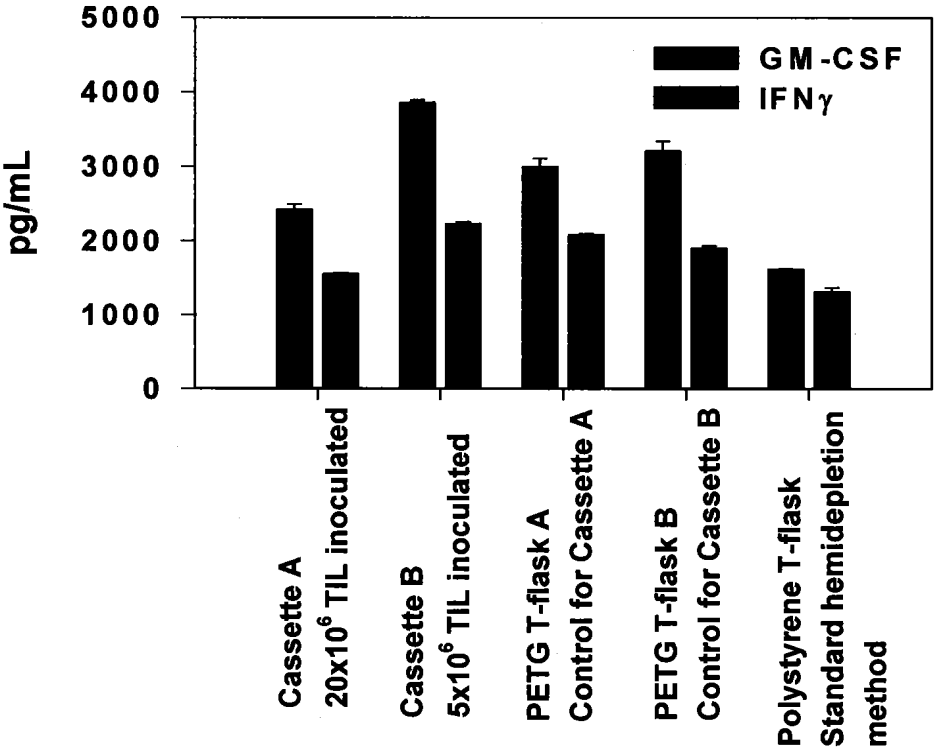
The undersigned declares further that all statements made herein are of his own knowledge are true and that all statements made on information are believed to be true. Further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of this application or any patent issuing thereon.

Signature Doug M. Smith

Date February 4, 2008

Figure 1. Functional activity and specificity of MART-1 reactive TILs produced under perfusion culture conditions in the clinical-scale bioreactor system, small-scale tissue culture flasks mimicking conditions used in the bioreactor system, or conventional control hemi-depletion tissue culture flasks. A) Cytokine release (IFNgamma and GM-CSF) in response to immobilized anti-CD3 mAb; B) Cytokine release (IFNgamma) in response to peptide loaded T2 cells or a panel of melanoma cell lines. Cytokine release is expressed as concentration in picograms per ml as determined by ELISA.

A.



B.